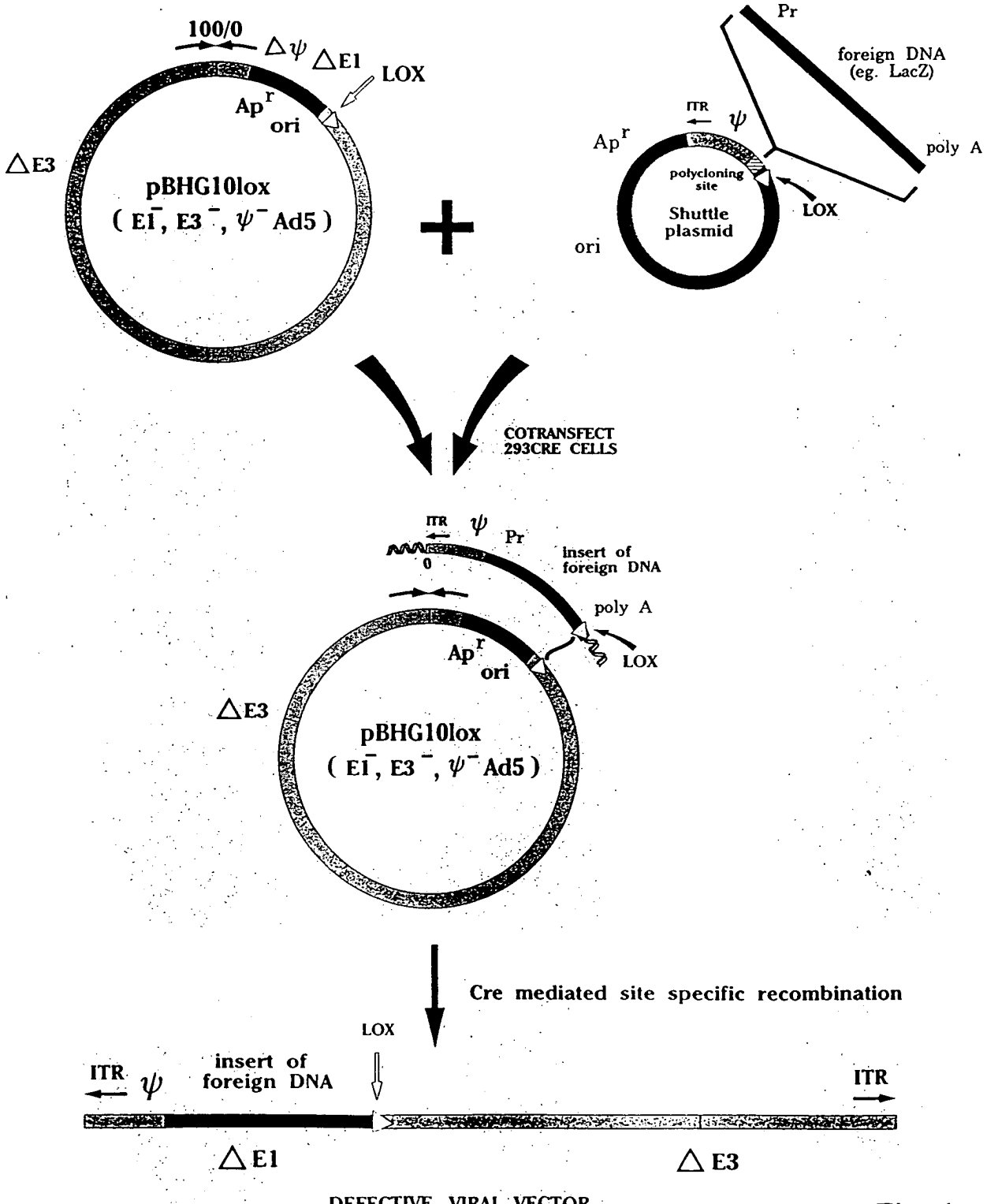
#6

Cotransfection of 293Cre cells with pBHG10lox and a "Lox" shuttle plasmid for generation of Ad expression vectors



DEFECTIVE VIRAL VECTOR

Fig. 1

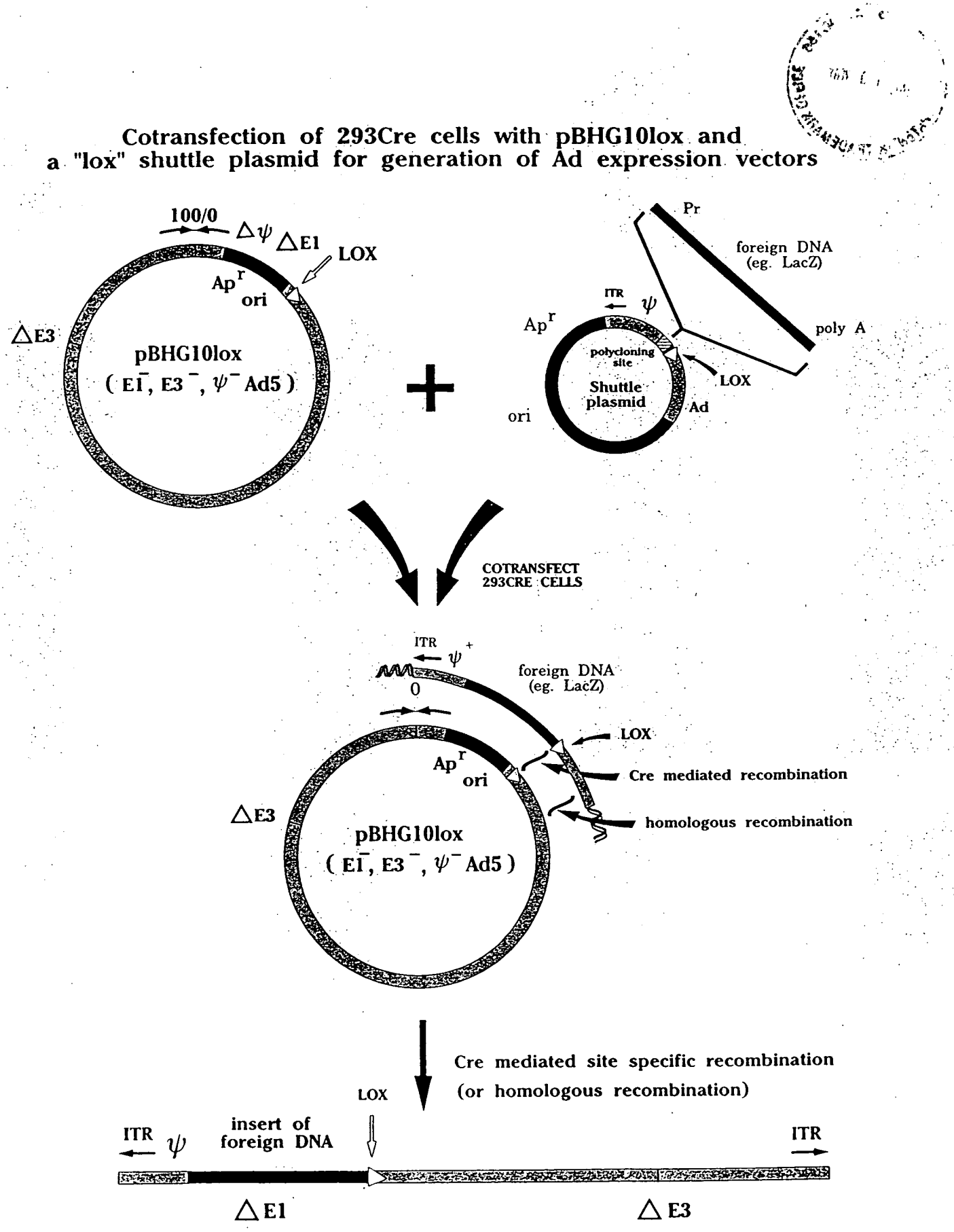

Cotransfection of 293Cre cells with pBHG10lox and a "lox" shuttle plasmid for generation of Ad expression vectors
100/0
Δψ
ΔE1
LOX
Ap^r
ori
ΔE3
pBHG10lox
(E1⁻, E3⁻, ψ⁻ Ad5)
Pr
foreign DNA
(eg. LacZ)
ITR
ψ
Ap^r
poly A
polycloning site
LOX
Shuttle plasmid
ori
Ad
COTRANSFECT 293CRE CELLS
ITR
ψ⁺
foreign DNA
(eg. LacZ)
0
LOX
Ap^r
ori
Cre mediated recombination
ΔE3
homologous recombination
pBHG10lox
(E1⁻, E3⁻, ψ⁻ Ad5)
Cre mediated site specific recombination
(or homologous recombination)
LOX
ITR
ψ
insert of foreign DNA
ITR
ΔE1
ΔE3
DEFECTIVE VIRAL VECTOR

Fig. 2

OLIGONUCLEOTIDES USED IN CLONING

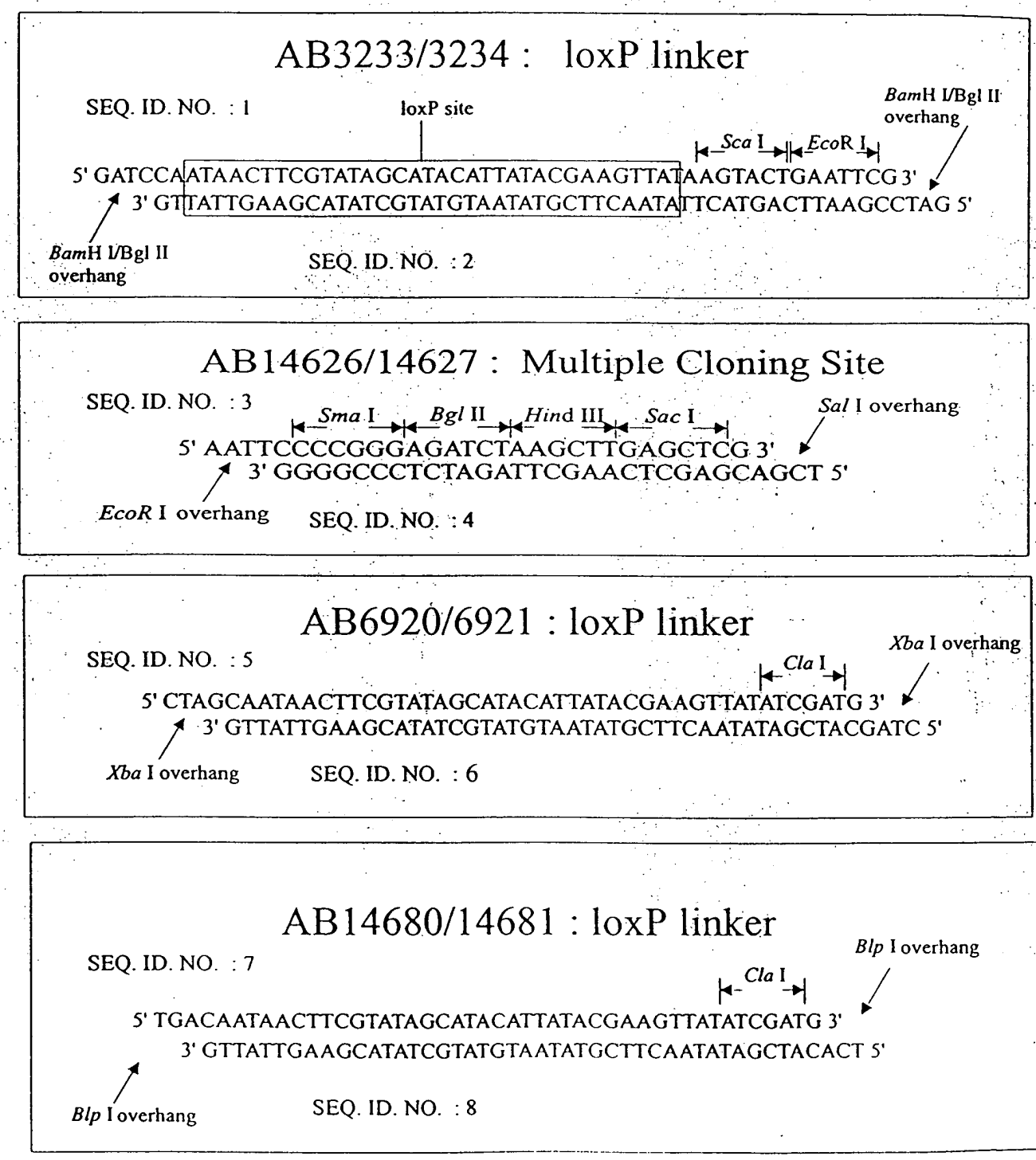


Fig. 3

CONSTRUCTION OF A CIRCULAR GENOMIC PLASMID FOR Ad VECTOR RESCUE USING THE Cre/ loxP SYSTEM

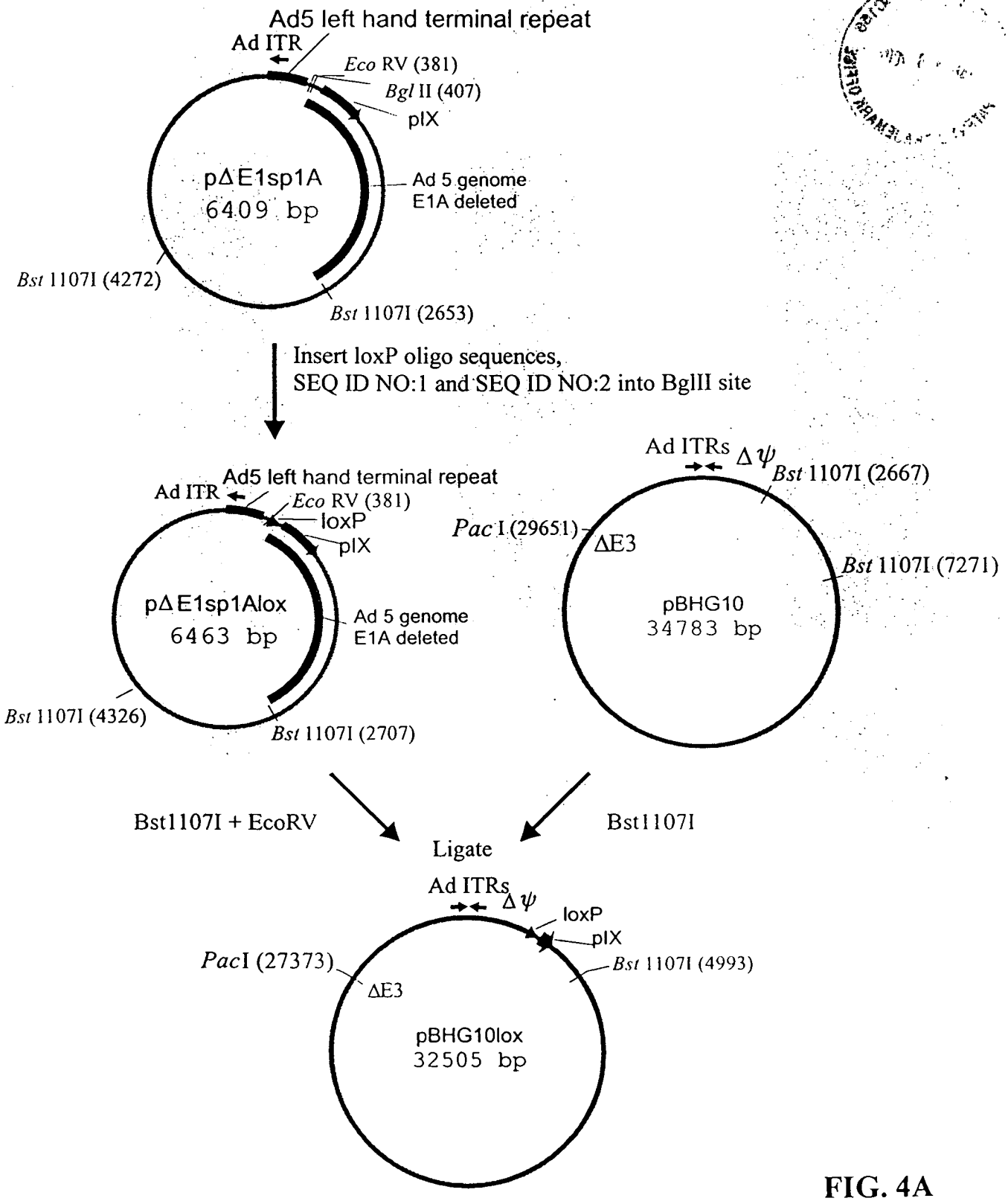


FIG. 4A

CONSTRUCTION OF pBHGdX1Plox

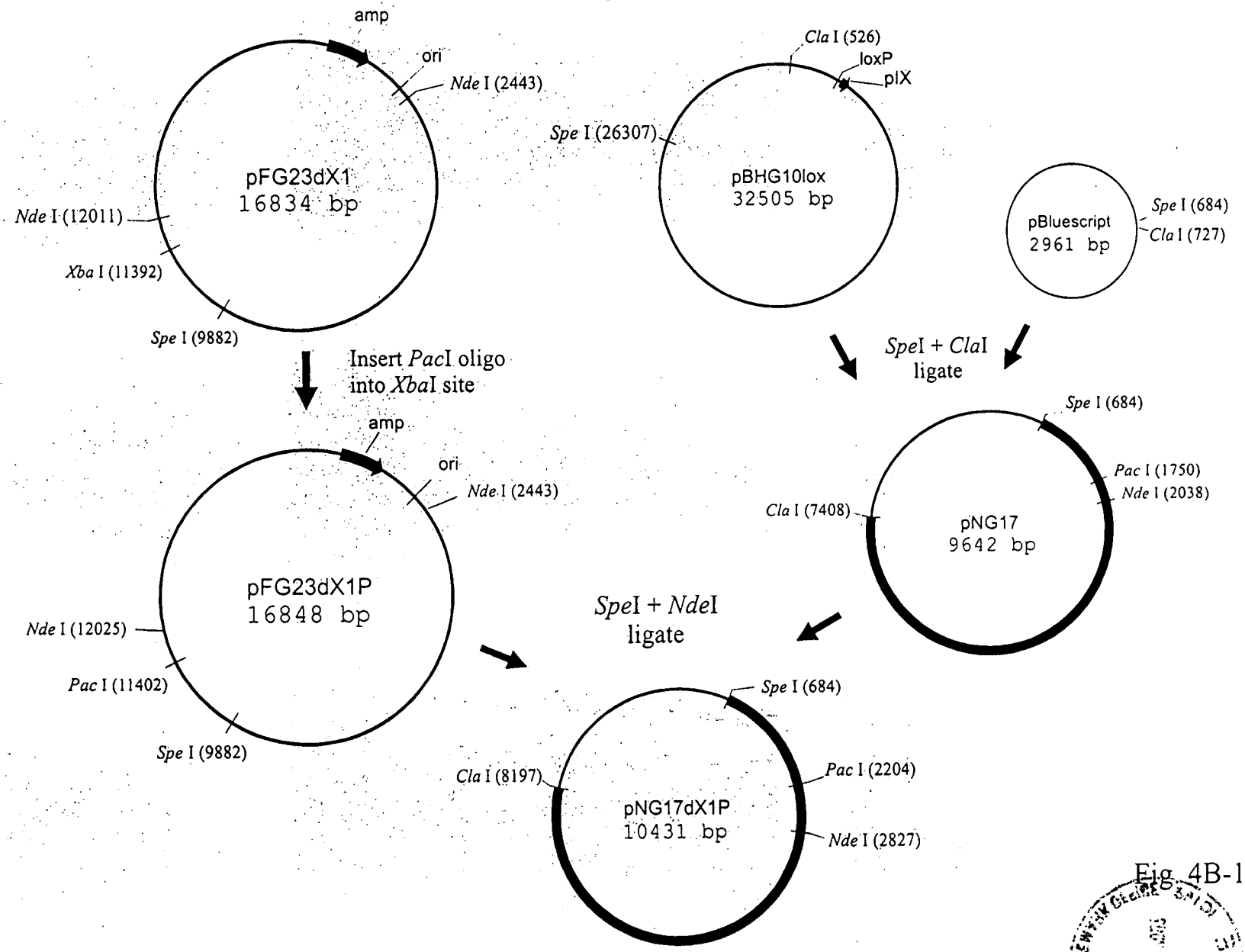


Fig. 4B-1

CONSTRUCTION OF pBHGdX1Plox

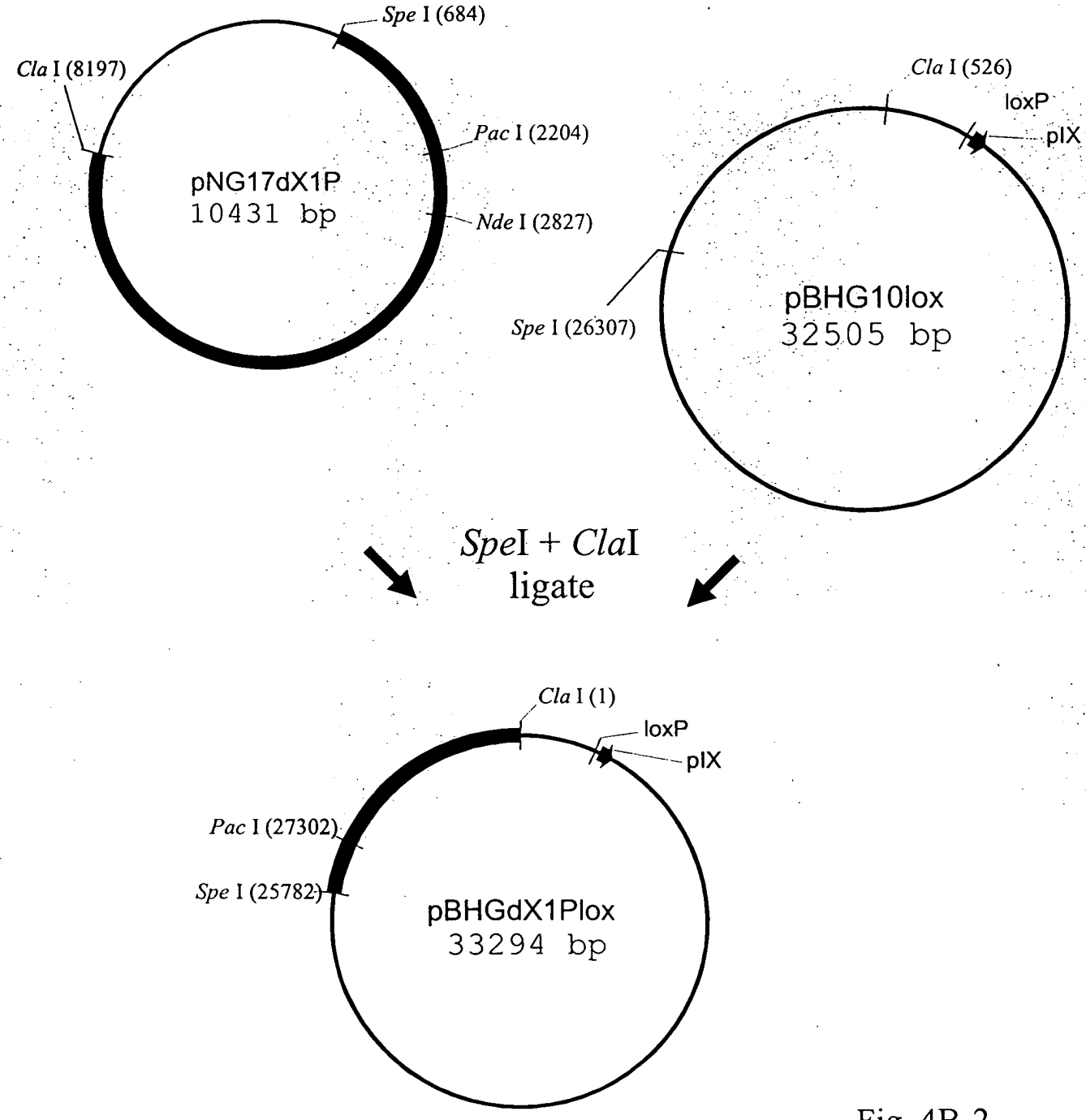


Fig. 4B-2

CONSTRUCTION OF pBHGE3lox

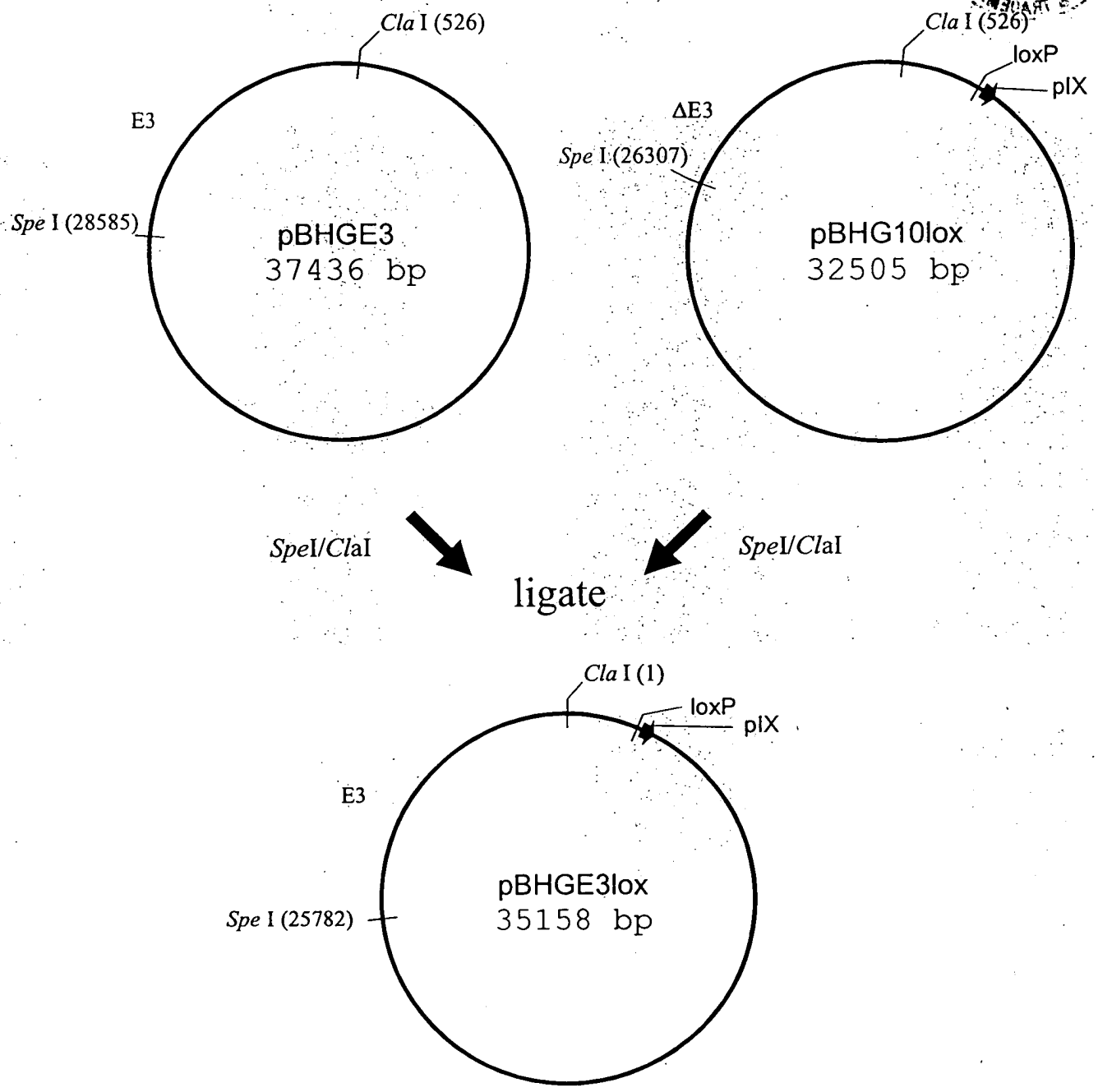


Fig. 4C

CONSTRUCTION OF pΔE1SP1A & pΔE1SP1B loxP PLASMIDS FOR RESCUE OF FOREIGN DNA

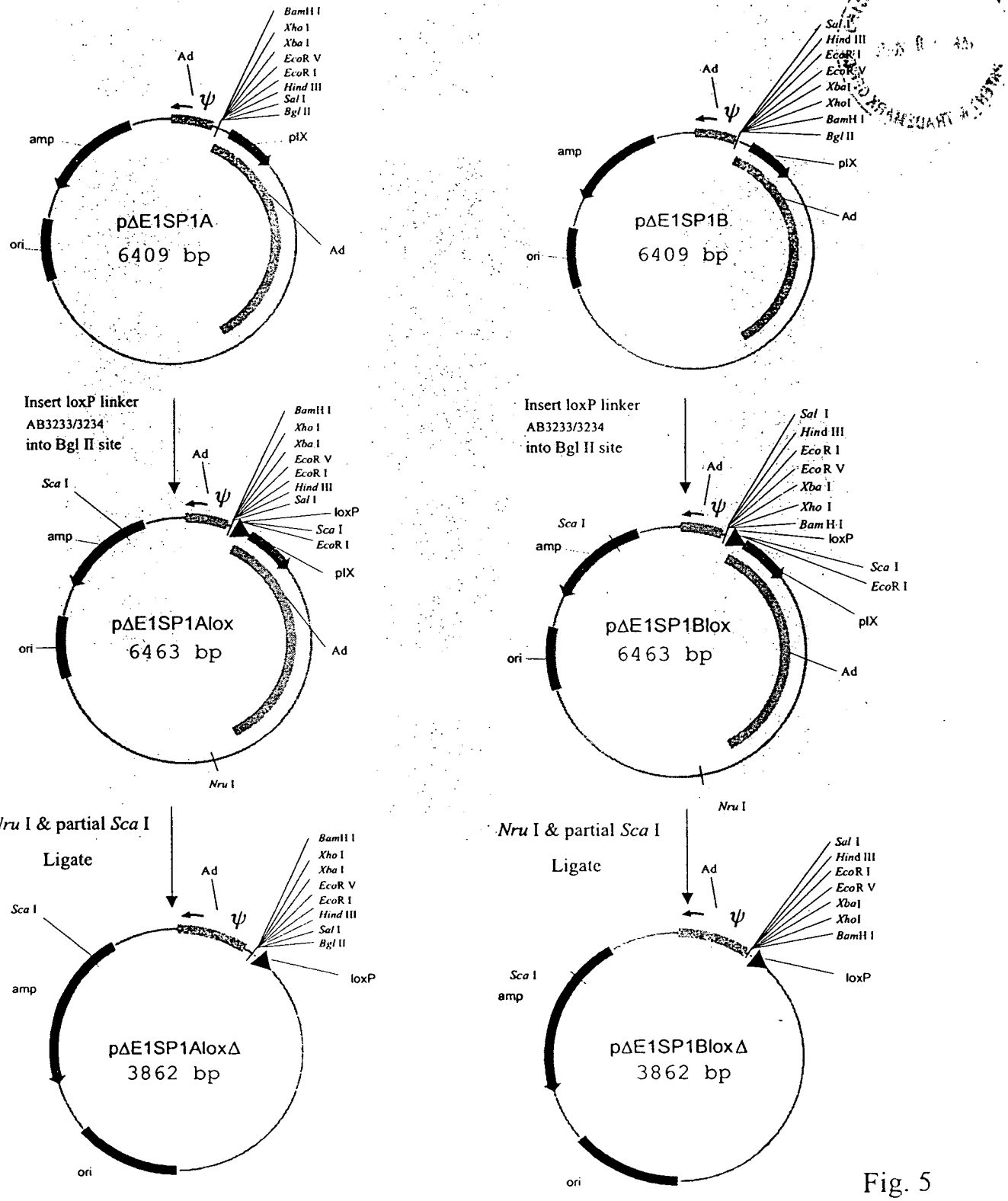


Fig. 5

CONSTRUCTION OF pMH4LOX, pMH4LOXΔ and pMH4LOXΔLINK SHUTTLE PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

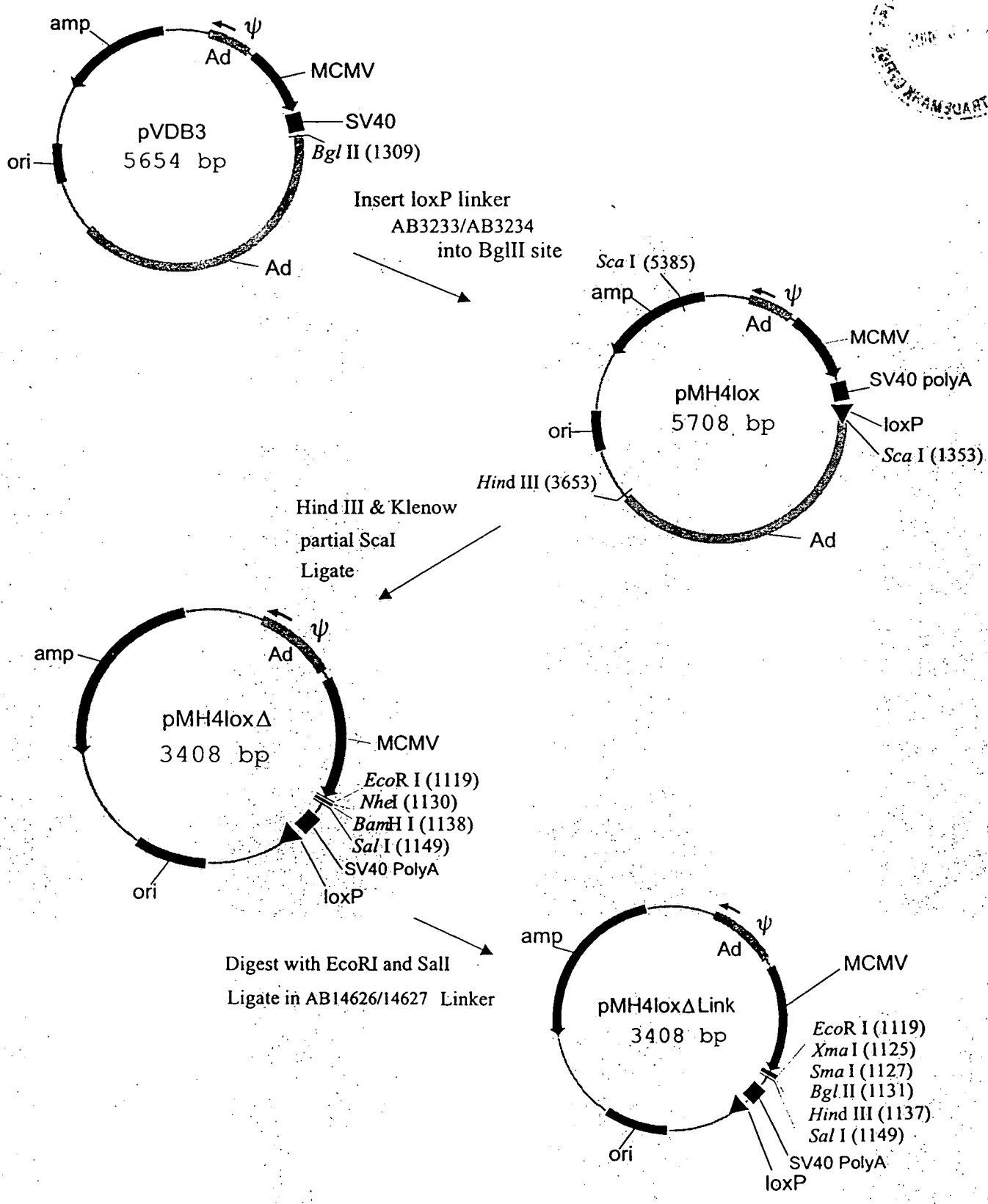


Fig. 6A

CONSTRUCTION OF A SHUTTLE PLASMID CONTAINING A pUC DERIVED ORIGIN

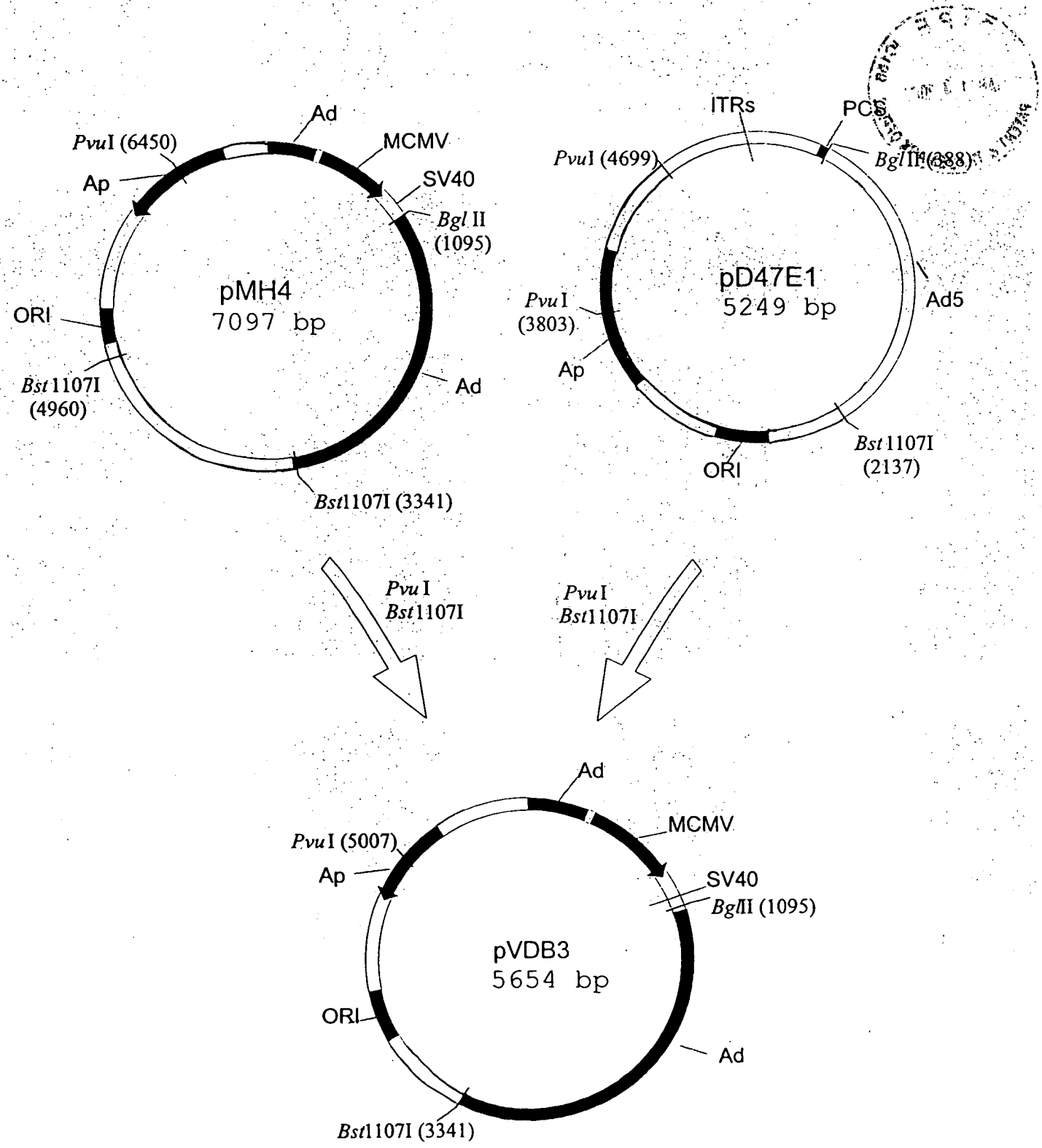


Fig. 6B

CONSTRUCTION OF HCMV loxP PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

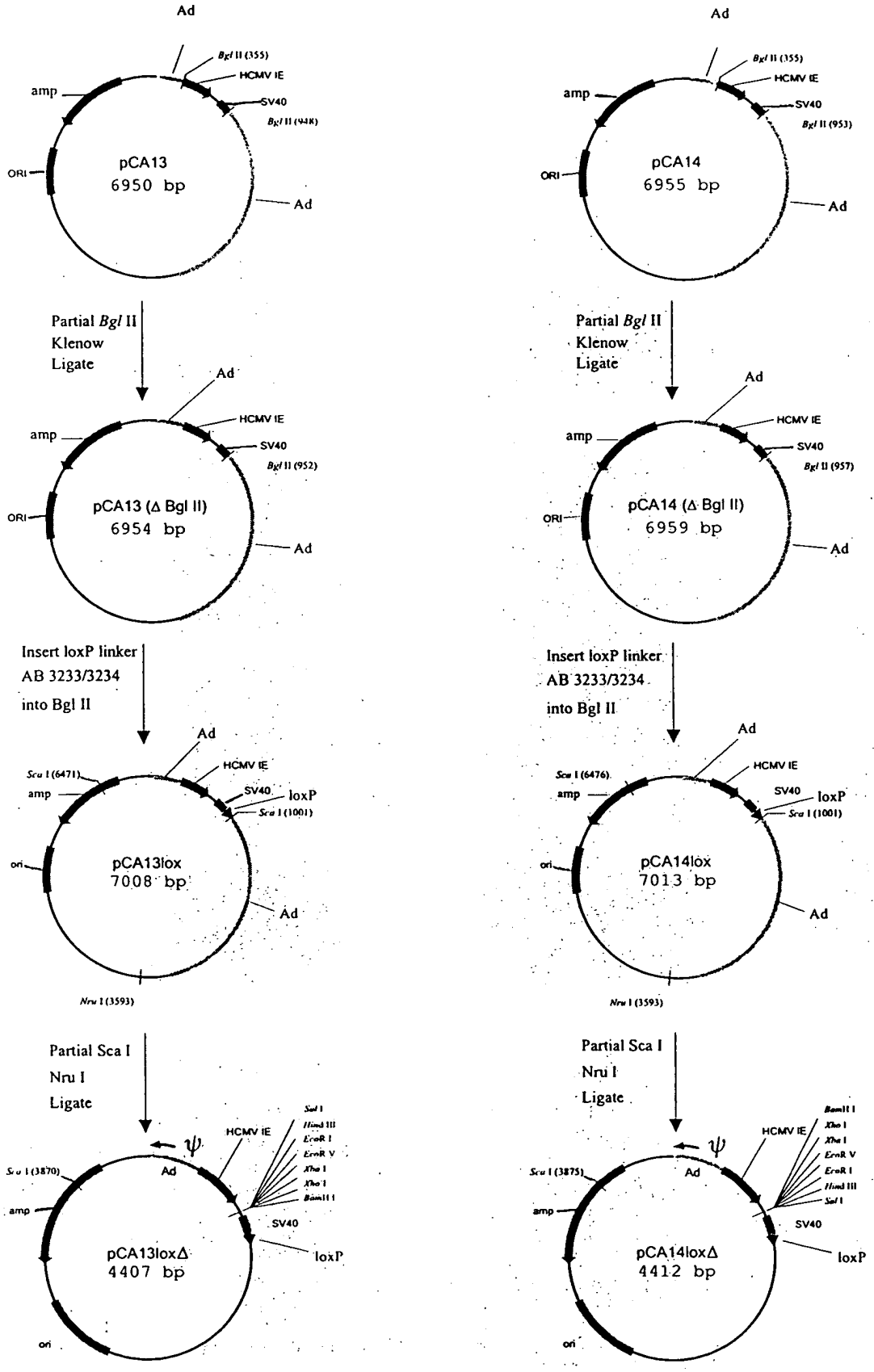


Fig. 7

CONSTRUCTION OF pCA36LOX and pCA36LOXΔ SHUTTLE PLASMIDS FOR RESCUE OF LACZ

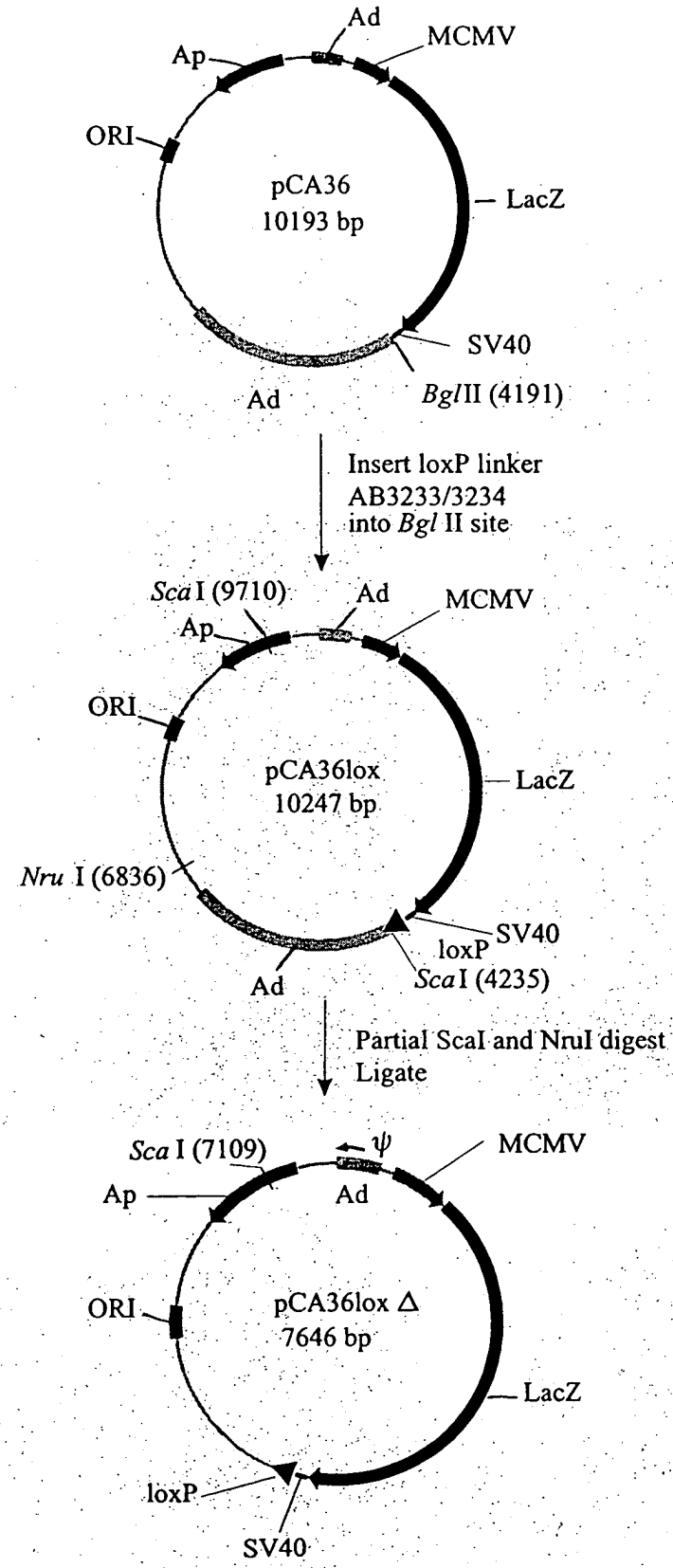
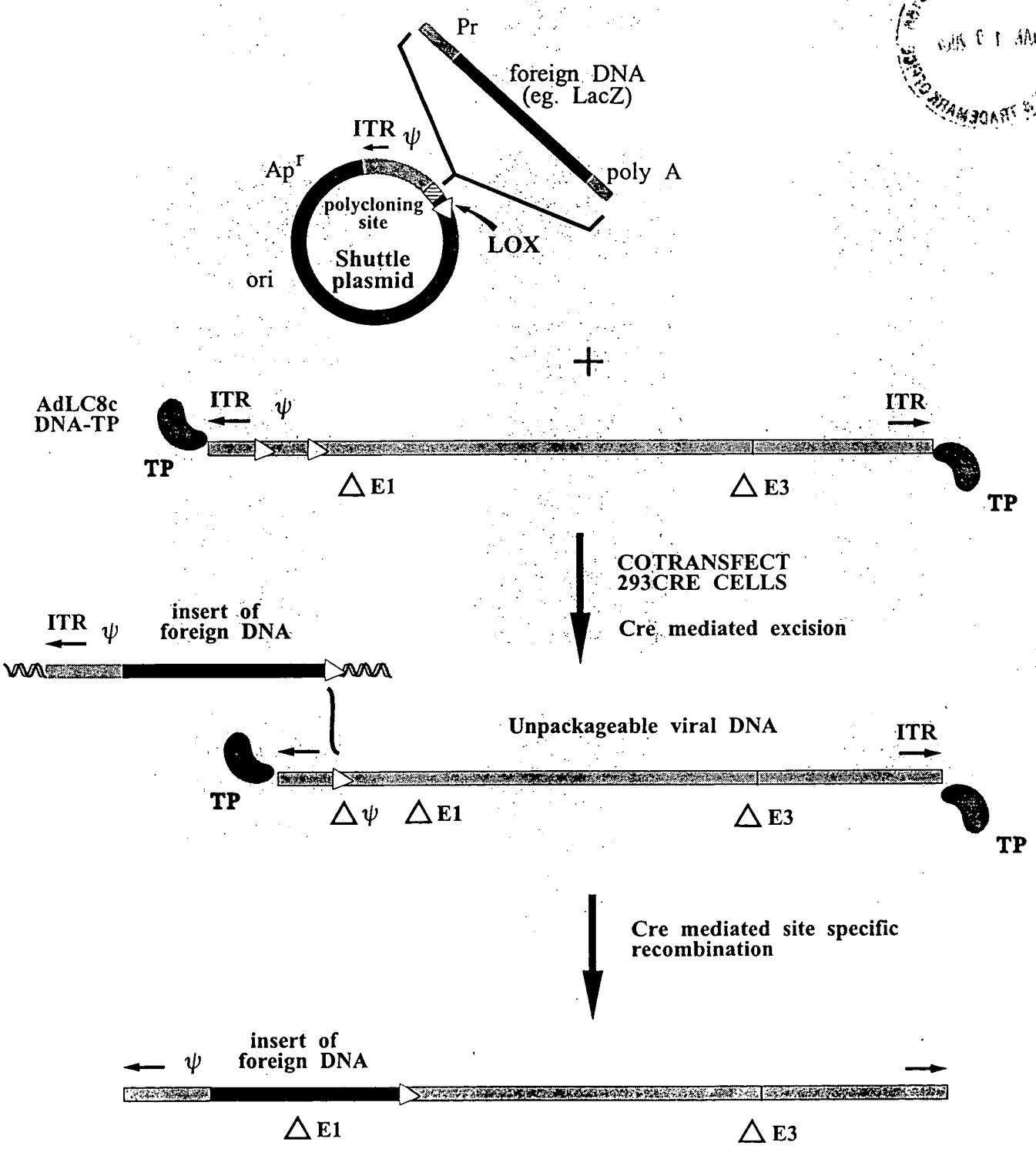


Fig. 8A

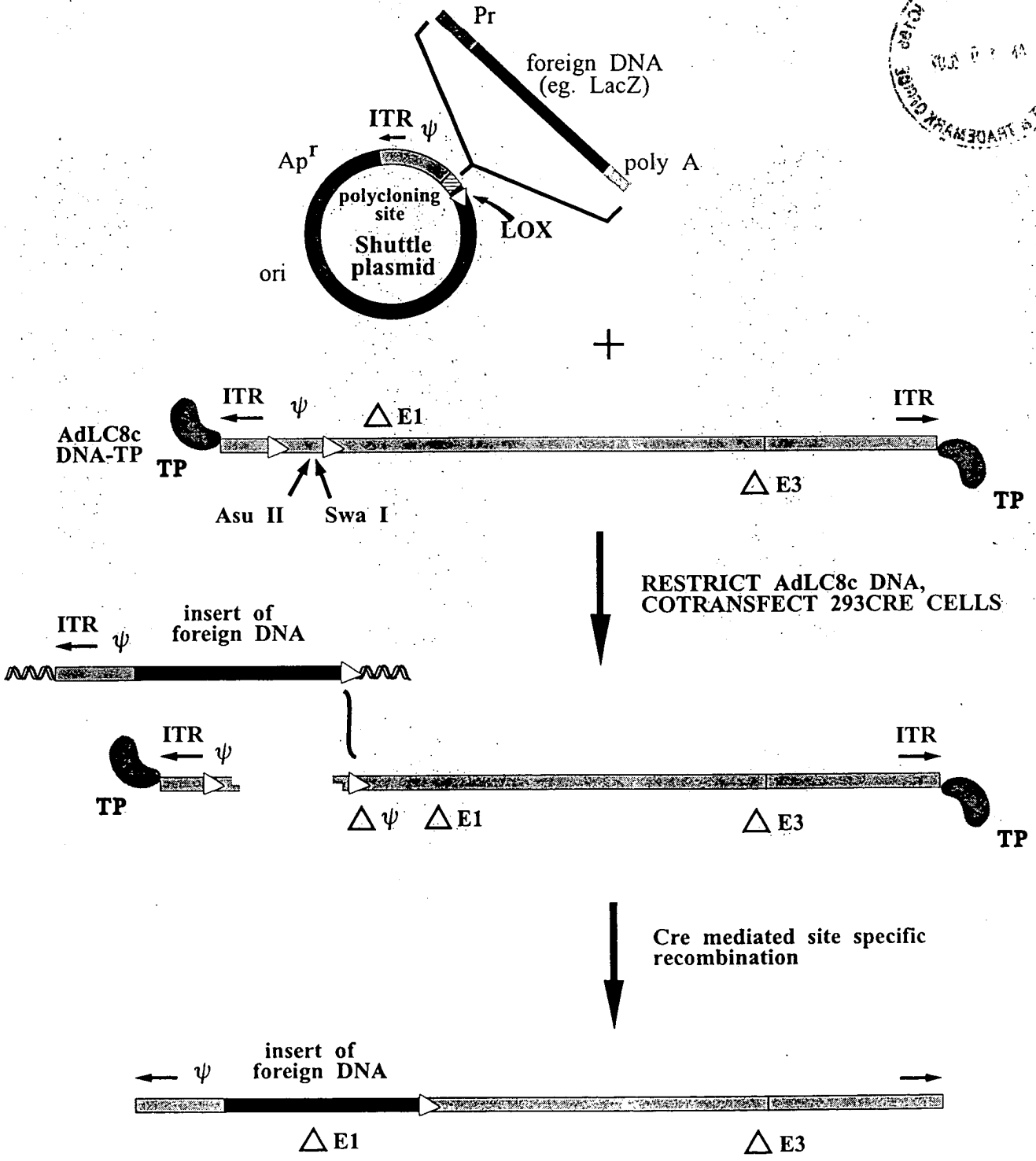
Cotransfection of 293Cre cells with AdLC8c DNA-TP and a shuttle plasmid containing a loxP site for generation of Ad expression vectors



DEFECTIVE VIRAL VECTOR

Fig. 8B

Cotransfection of 293Cre cells with restricted AdLC8c DNA-TP and loxP shuttle plasmid for generation of Ad expression vectors



DEFECTIVE VIRAL VECTOR

Fig. 8C

CONSTRUCTION OF SHUTTLE PLASMIDS EXPRESSING Cre

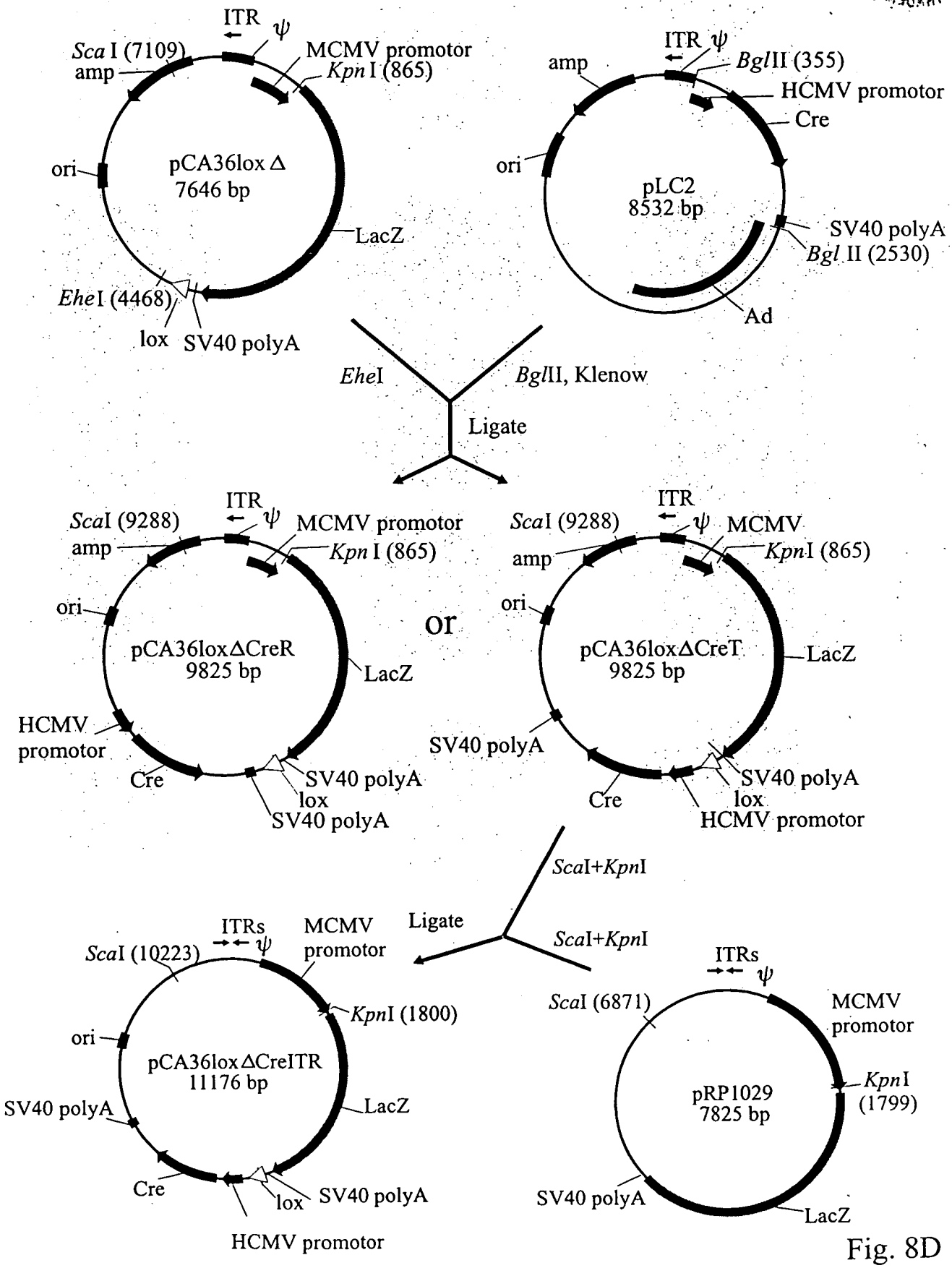
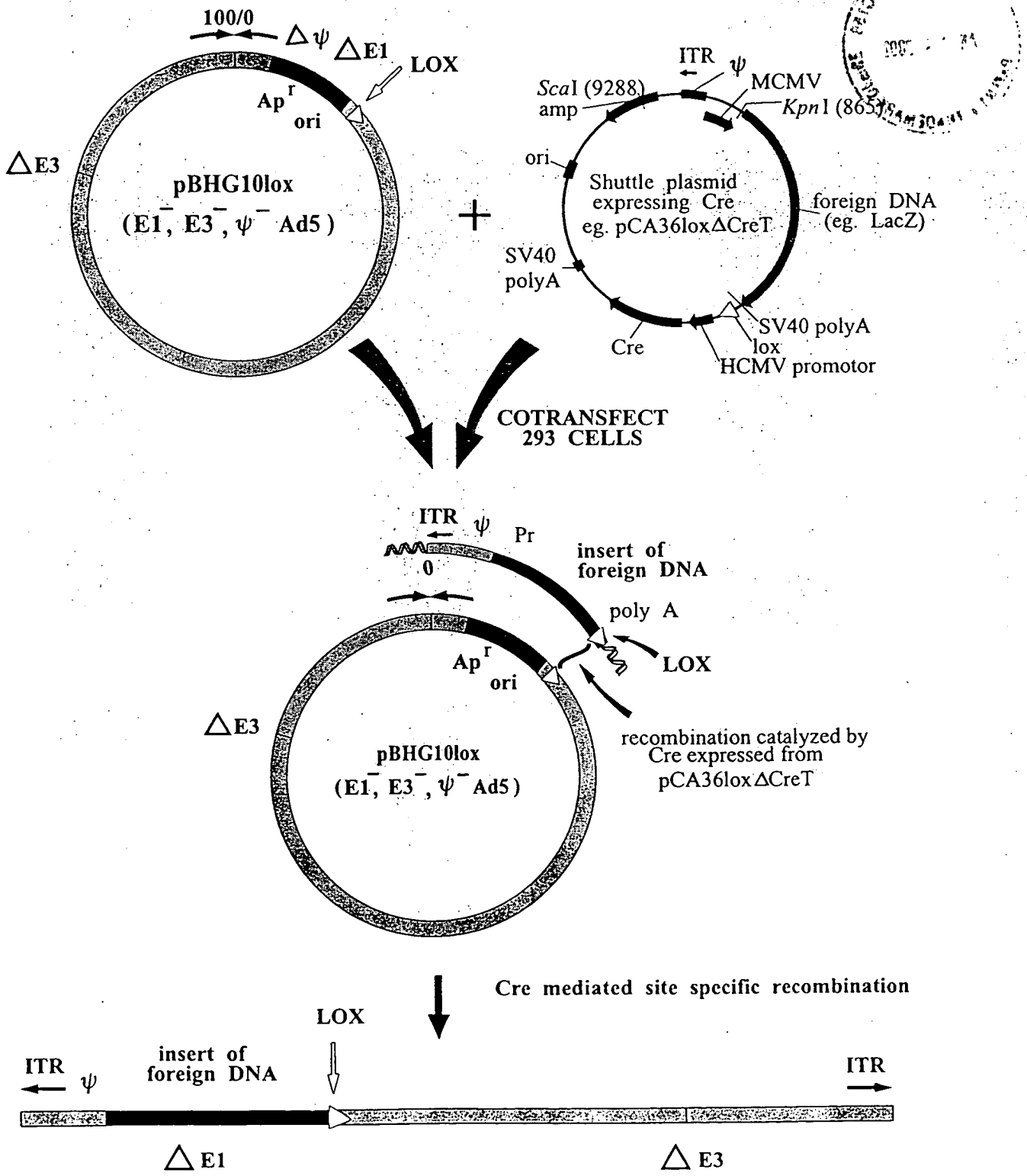


Fig. 8D

Cotransfection of 293 cells with pBHG10lox and a "Lox" shuttle plasmid expressing Cre for generation of Ad expression vectors



DEFECTIVE VIRAL VECTOR

Fig. 8E

CONSTRUCTION OF Ad GENOMIC PLASMID ENCODING CRE

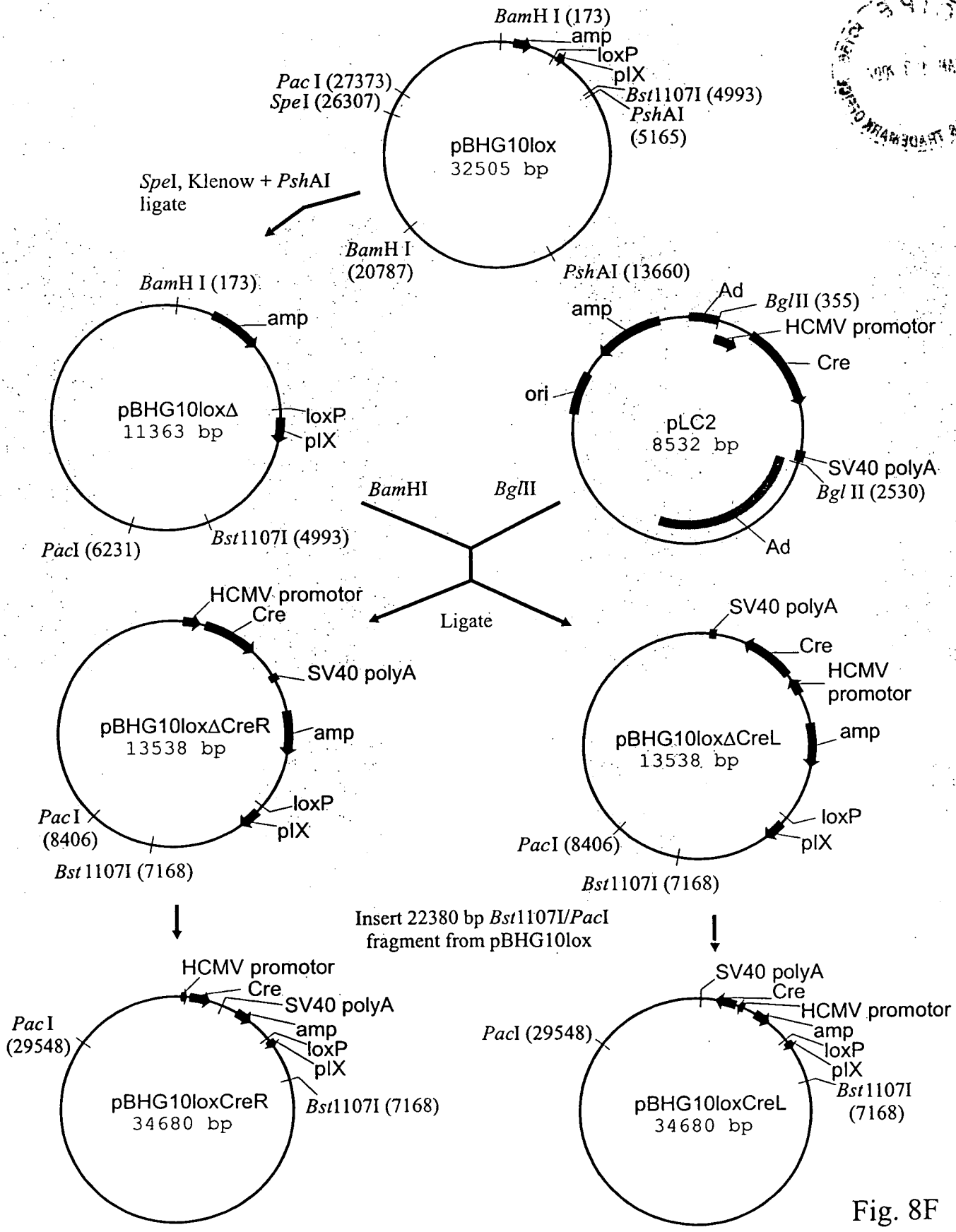
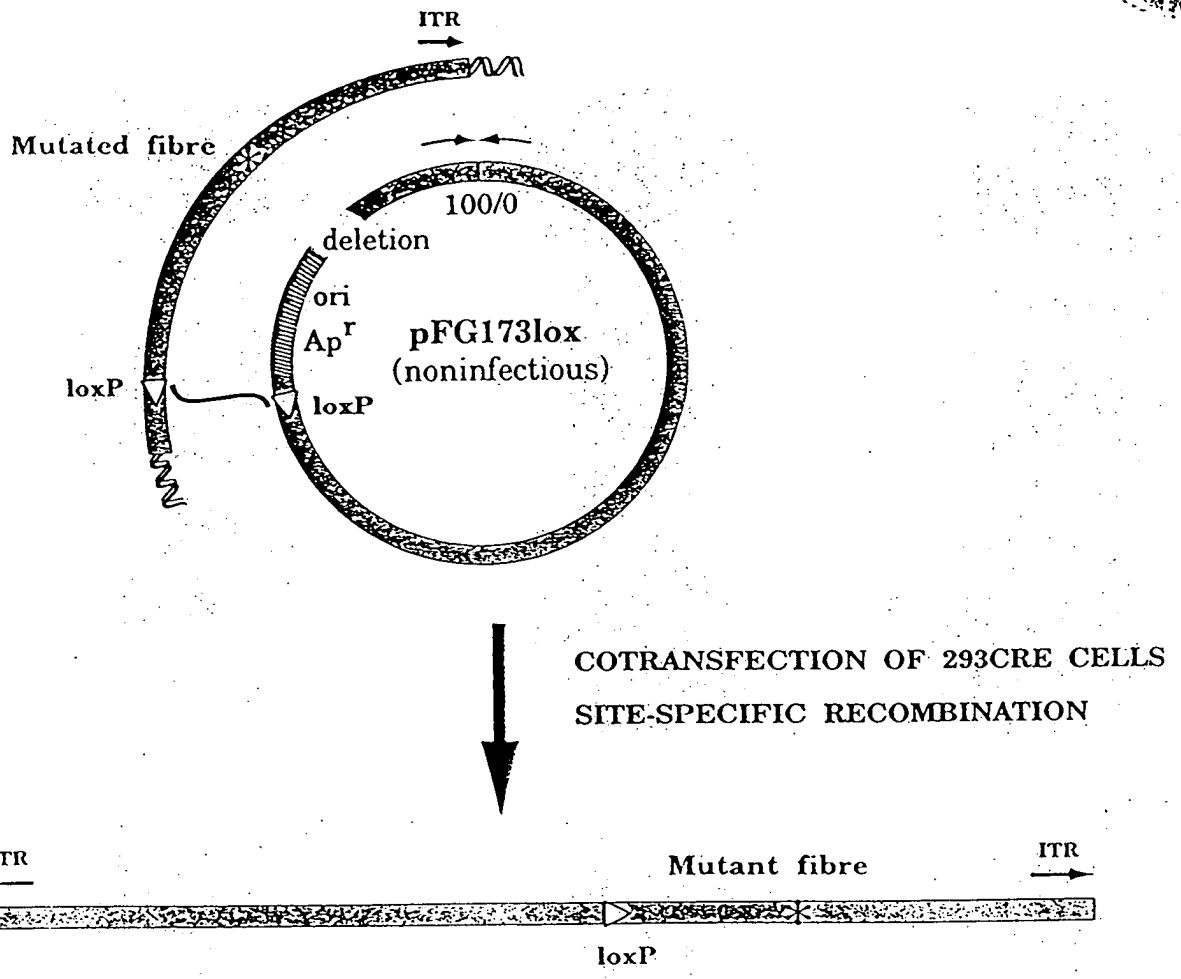


Fig. 8F

RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION



NONDEFECTIVE ($E1^+$) VIRUS WITH MUTATED FIBRE GENE

FIGURE 9A

CONSTRUCTION OF pAB14lox △

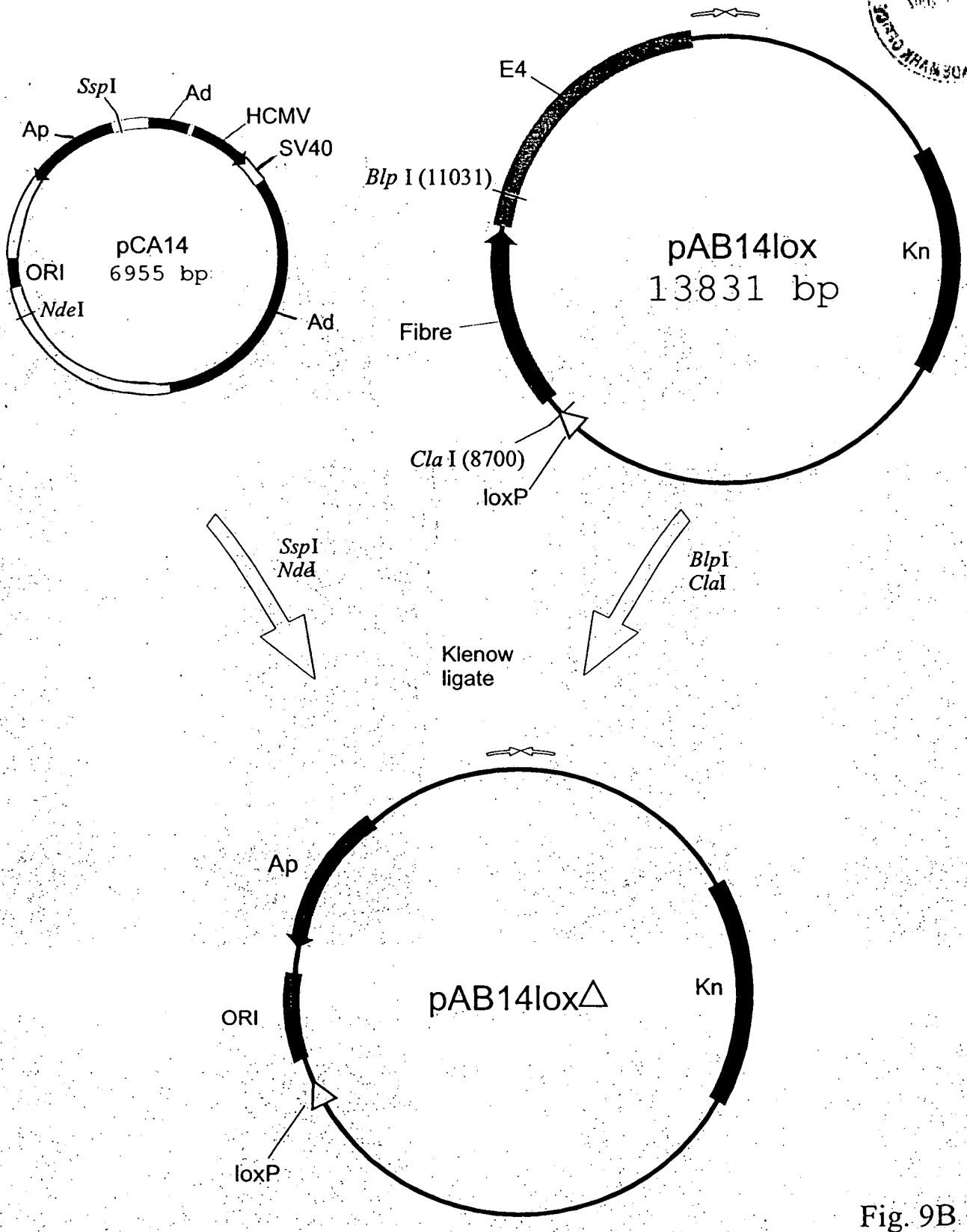
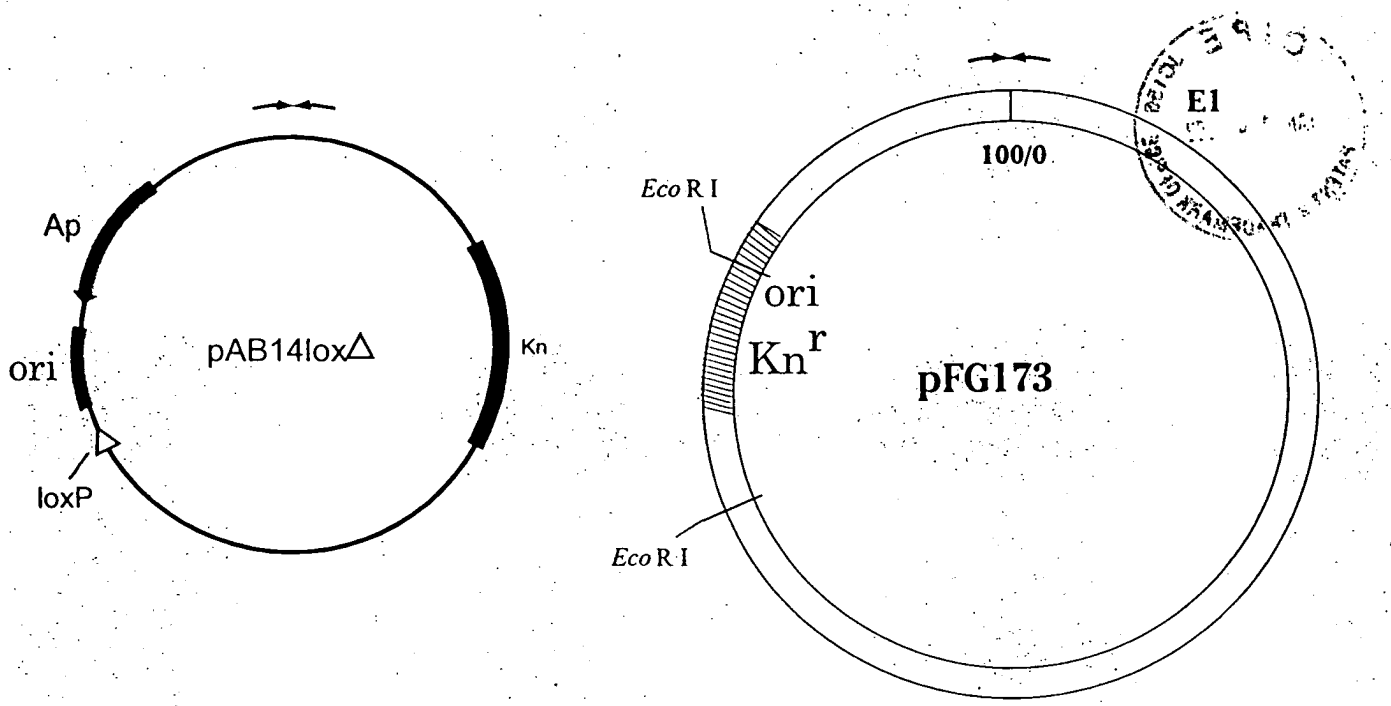


Fig. 9B

CONSTRUCTION OF pFG173lox



Restriction, transformation of E. coli, homologous recombination

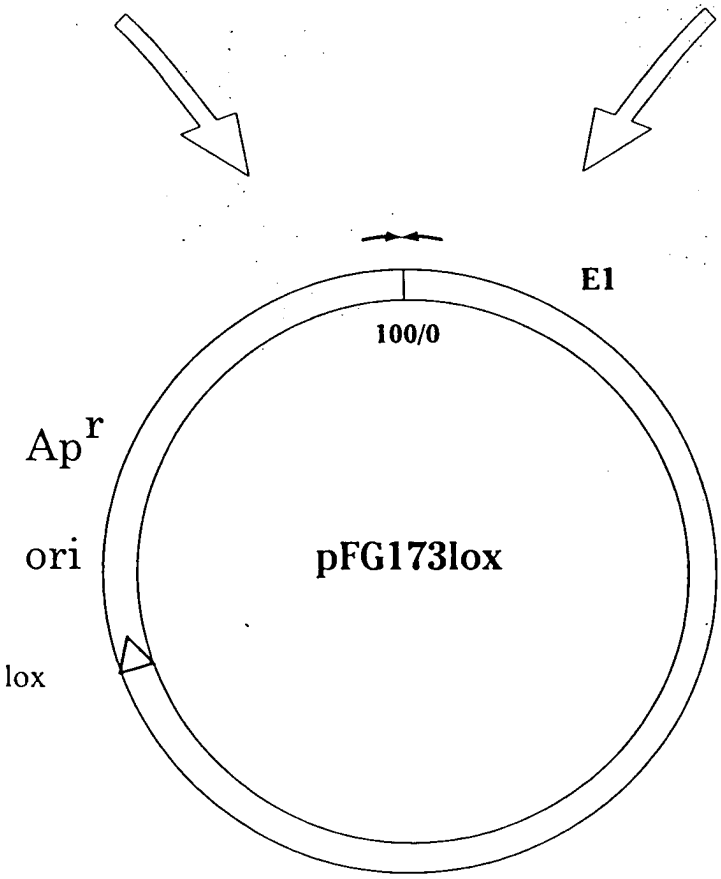


Fig. 9C

CONSTRUCTION OF pFG23dX1lox AND pFG23dX1loxc FOR RESCUE OF MUTANT FIBRE INTO AD VIRUS

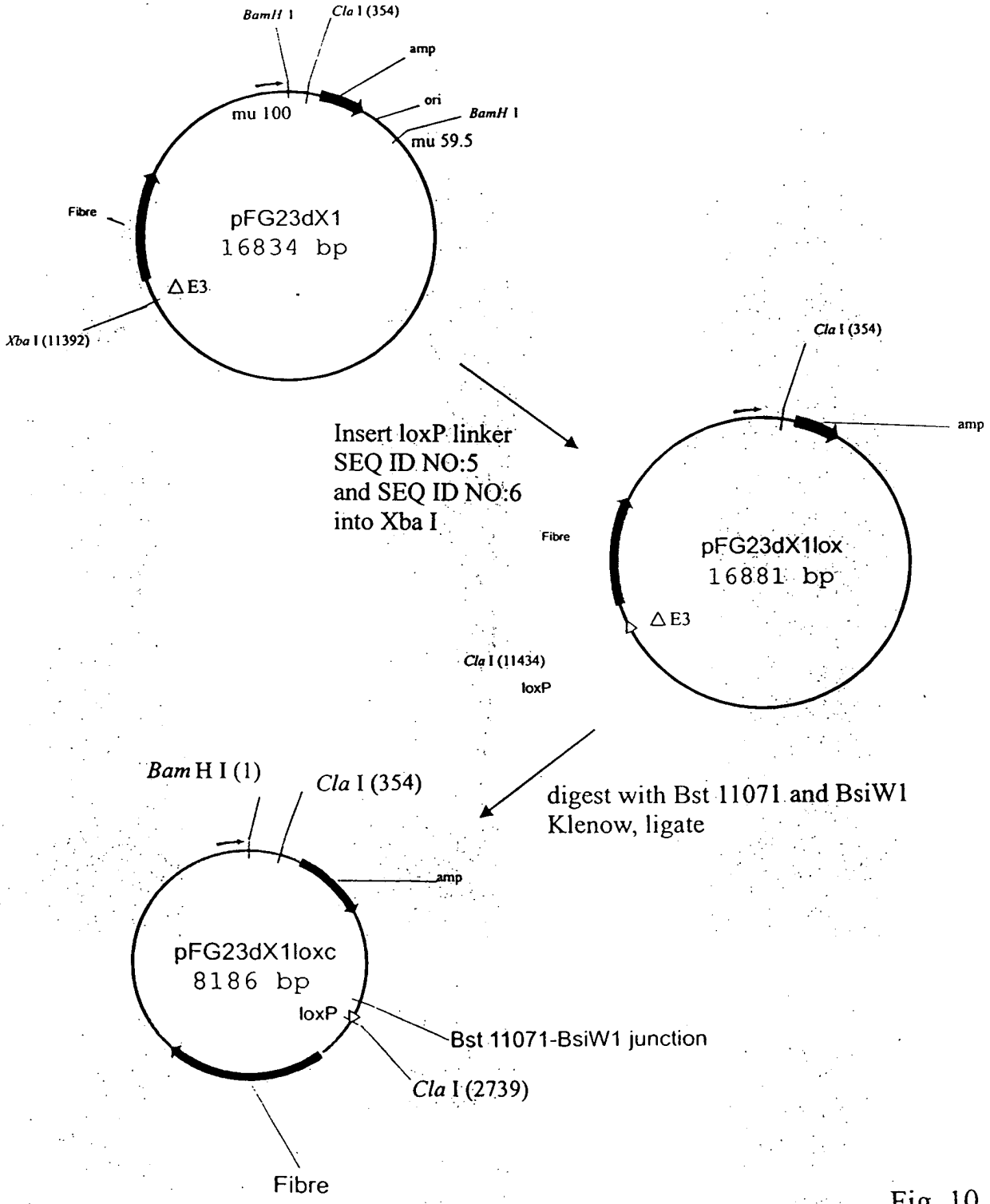


Fig. 10

A PLASMID FOR RESCUE OF A FOREIGN DNA INTO AD VIRUS

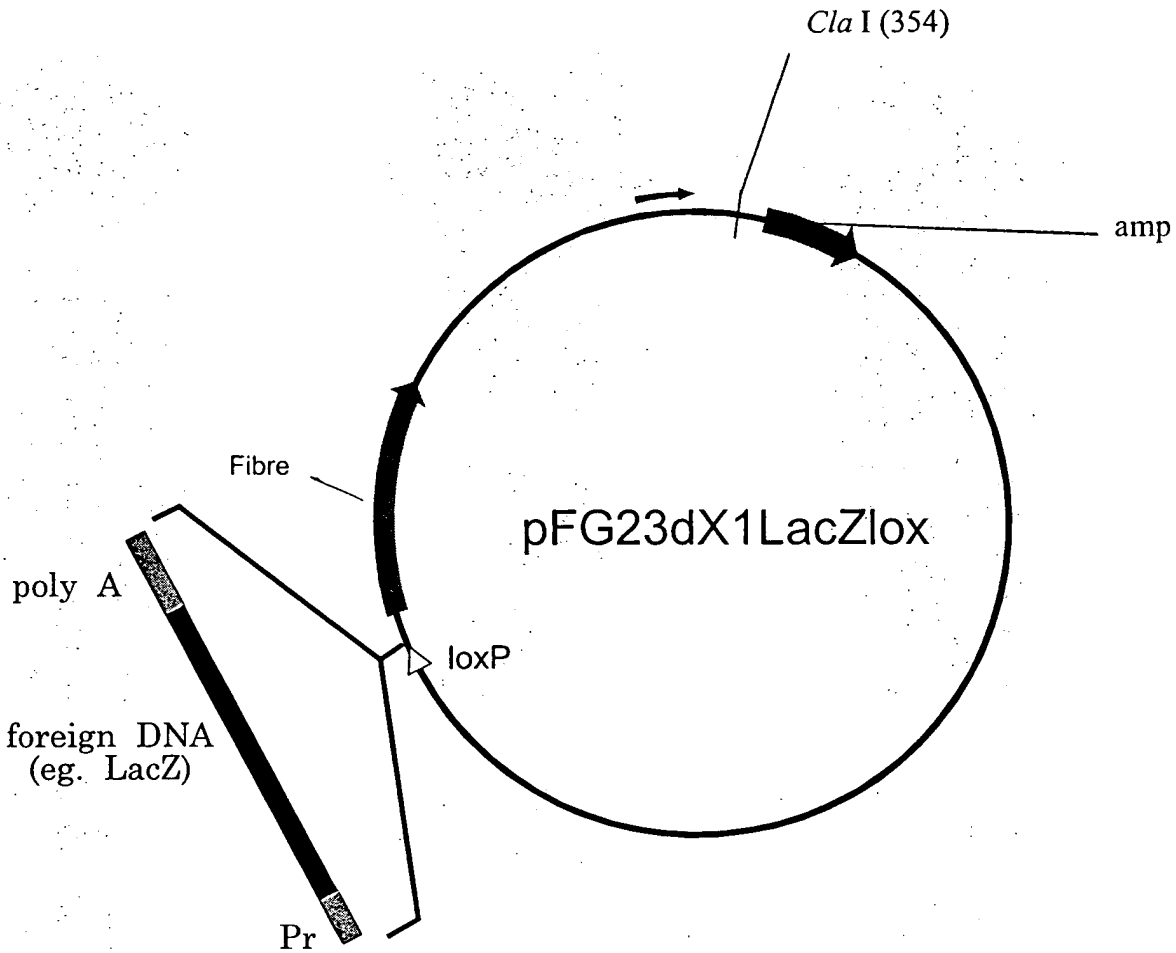


Fig. 11

Isolation of a virus containing a mutant fibre gene by Cre-lox recombination using DNA-TP and cotransfection

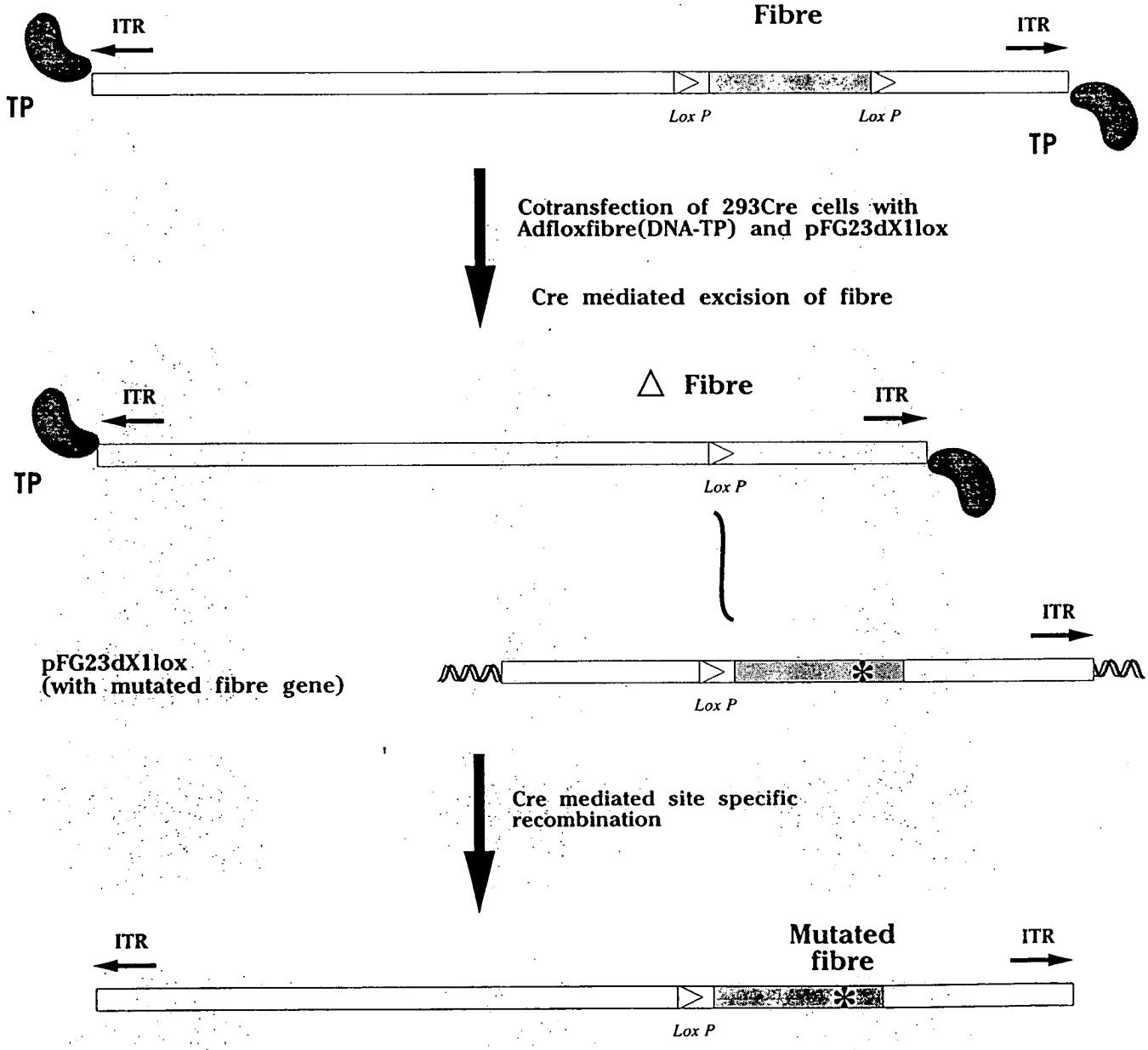
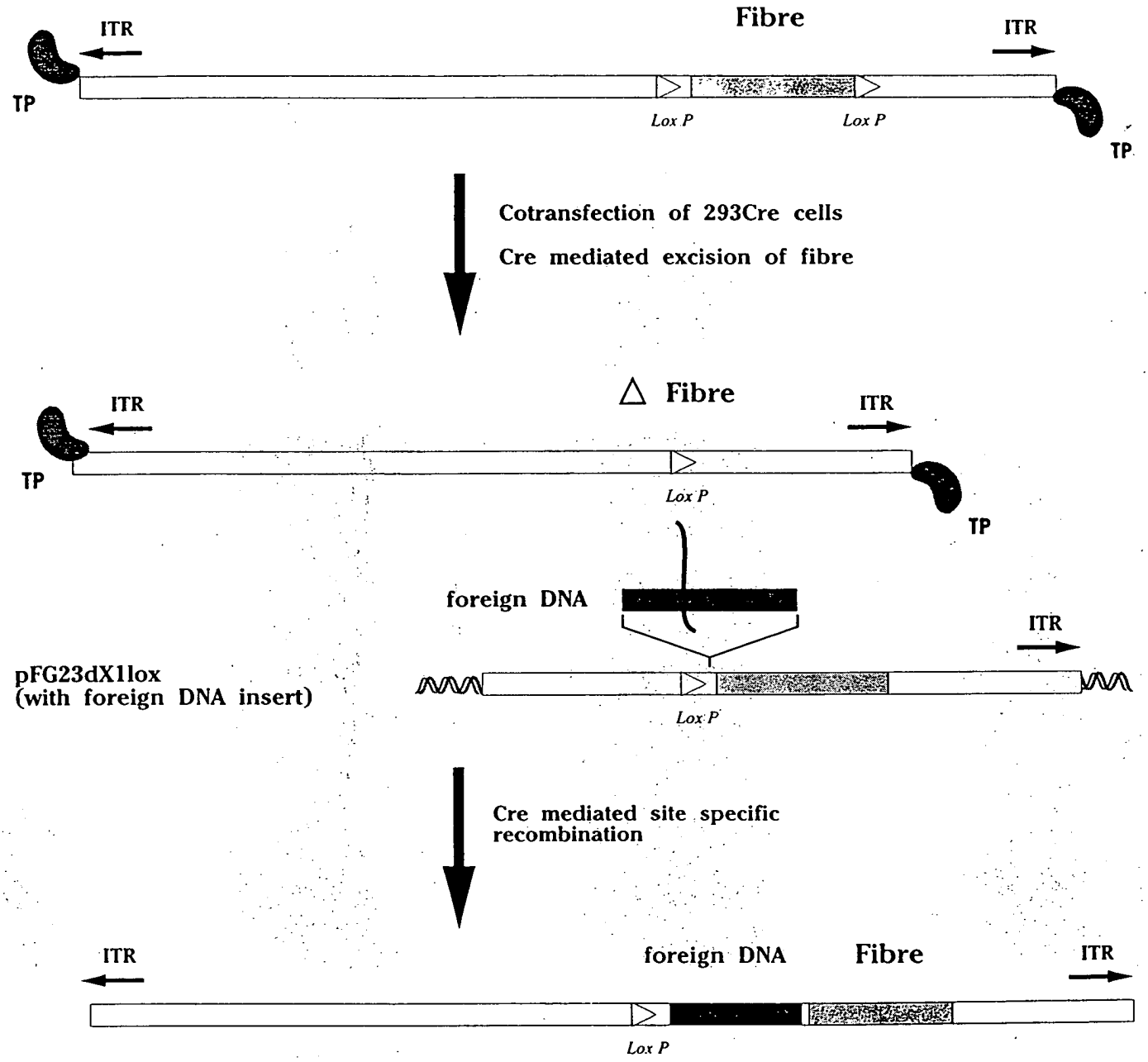


Fig. 12

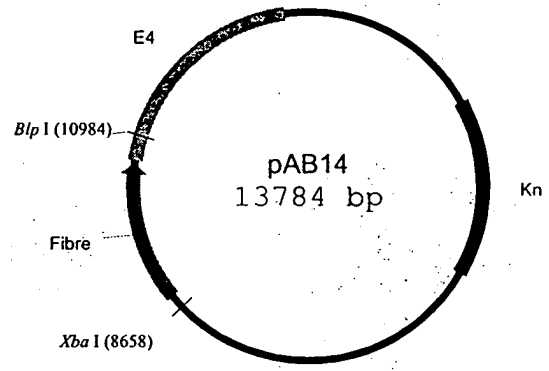
Isolation of a virus containing a foreign DNA insert upstream of the fibre gene by Cre-lox recombination



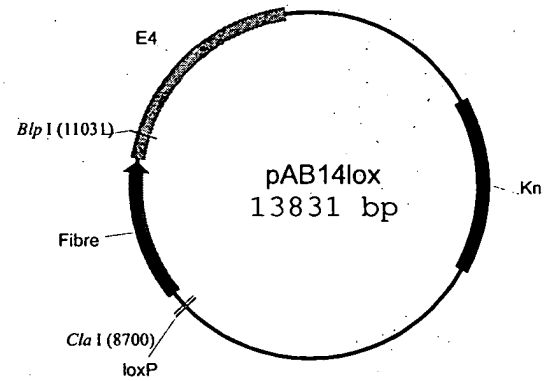
RECOMBINANT VIRUS CONTAINING AN INSERT OF FOREIGN DNA UPSTREAM OF THE FIBRE GENE

Fig. 13

CONSTRUCTION OF pAB14FL0X FOR ISOLATION OF AN AD VIRUS WITH A FLOXED FIBRE GENE



Insert loxP linker
AB6920/AB6921
into Xba I site



Insert loxP linker
AB14680/AB14681
into Blp 1 site

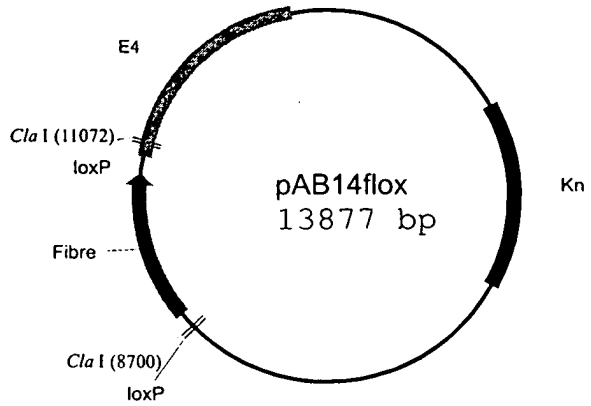
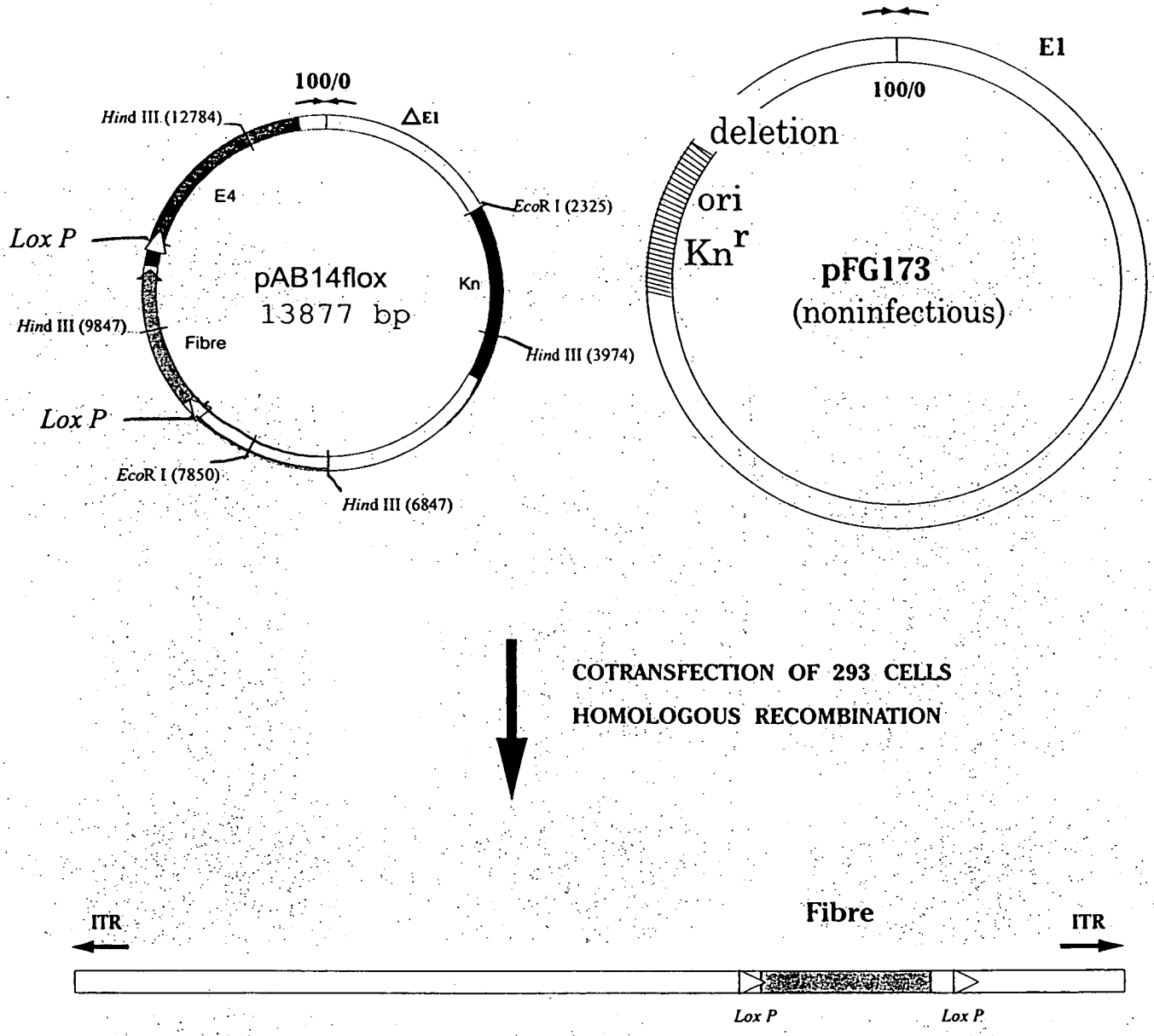


Fig. 14

Isolation of a virus containing a fibre gene with flanking lox P sites.



NONDEFECTIVE ($E1^+$) VIRUS (ADFLOXFIBRE) CONTAINING A FLOXED FIBRE GENE

Fig.15